Search for the theraphy resistant phenotype in lung cancer

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Summary

This thesis contains a number of studies in which clinical data obtained from a series of lung cancer patients at diagnosis, during treatment and the development of drug resistance have been collected. A series of new parameters obtained from biopsy specimens, marrow aspirates, and biopsy specimen derived cell lines, was collected in these patients. It was attempted to correlate these laboratory findings with the clinical information.

In chapter I a method is described enabling the routine assessment of phenotypic differences using immunohistochemistry, in small lung cancer biopsy specimens. This can be done without interfering with the normal histopathological evaluation of the case. This is important because the WHO classifications of lung cancer remains the reference point to which monoclonal antibody based subclassifications should be compared.

To distinguish bronchial carcinoid tumors and small cell lung cancer may be very difficult using clinical, cytopathological and/or biochemical parameters alone. Chapter II describes two patients who were initially judged to suffer from small cell lung cancer. Clinical data cast doubt upon this diagnosis, and in one patient a very long natural history, exeeding 32 years, could be reconstructed. Small cell lung cancer, atypical carcinoid and carcinoid all share a series of neuroendocrine differentiation features but may behave completely different.

In Chapter III the results are described of giving a reinduction chemotherapy with the induction regimen. In most SCLC patients a remission of the tumor can be induced by short term (5 cycles) polychemotherapeutic treatment with cyclophosphamide, etoposide and doxorubicin. The duration of the remission seldomly exeeds 2 years. These results can not be improved by increasing the number of treatment cycles given. It is generally assumed that the effect of a reinduction polychemotherapeutic treatment in relapsing small cell lung cancer is minimal because no non-cross resistant drug schedules are available. We have treated relapsing small cell lung cancer, with the same combination of drugs which was used in the induction therapy. This resulted in a second response in 23 out of 37 patients. Factors influencing the occurrence of a second relapse were: A complete response after the first five cycles , and a first response duration > 34 weeks.

A detailed series of *in vitro* studies have been described in Chapter IV. Three small cell lung cancer (SCLC) cell lines have been established from one patient during longitudinal follow-up. During this period the tumor changed from sensitive to completely resistant to (chemo)therapy. A phenotypical and functional characterization of the different cell lines is given in combination with the matching clinical data. *In vitro* parameters which were proposed to reflect the development of drug resistance did not changes in these cell lines. The expression of neuroendocrine markers, as detected by

monoclonal antibodies was slightly decreased in both recurrent disease biopsy specimens and cell lines.

In Chapter V it is shown that, for the detection of metastasis in bone marrow of patients with small cell lung cancer, immunofluorescence with a monoclonal antibody detecting a SCLC related membrane antigen (MOC 1) may be used. This procedure was performed on 53 bone marrow aspirates obtained from 30 patients. In 63% of the patients MOC-1 reactive cells were detected. Simultaneous histopathological examination of the bone marrow biopsies detected tumor cells in 20% of the cases. Especially for the selection of patients who might benefit from an adjuvant radio and/or surgical therapy this procedure may be rewarding.

Chapter VI inventarizes the expression of intermediate sized filament proteins cytokeratin 10 and cytokeratin 18 using a monoclonal antibody guided immunohistological method. This to detect subgroups within small cell lung cancer that might have clinical relevance. Reactivity with the antibodies was subsequently correlated to clinical data. An increased expression of cytokeratin 18 was observed in metastatic SCLC lesions. In this context it is of interest that cytokeratin 18 is not expressed in the "variant" type of small cell lung cancer, the proposed *in vitro* counterpart of therapy resistant small cell lung cancer.

In Chapter VII the results of staining biopsy-specimens with a panel of monoclonal antibodies, directed against antigens, related to a neuroendocrine and epithelial differentiation state, are presented. This panel was prospectively applied to a series of small cell lung cancer specimens obtained before the induction polychemotherapy. No differences in panel reactivity were noted between partial and complete responders. Neuroendocrine differentiation antigens were not expressed in two patients who failed to respond to the induction polychemotherapeutic regimen. A third SCLC specimen without neuroendocrine differentiation antigens, was obtained from a small cell lung cancer patient in whom a post surgical pathological staging revealed no metastasis. This indicates that SCLC without neuroendocrine differentiation antigens contains a group of tumors clinically mimicing non-SCLC.

Chapter VIII describes the neuroendocrine differentiation in a series SCLC tumor biopsy specimens obtained either before induction chemotherapy, or, at relapse. It was found that the overall expression of neuroendocrine differentiation antigens was decreased in the recurrent disease group. This decrease was especially clear for the antigens recognized by the monoclonal antibodies MOC-21 and MOC-32. When the recurrent disease group was further subdivided into cases either responding or non-responding to subsequently given reinduction chemotherapy, it became apparent that the observed decrease in neuroendocrine antigen expression was largely confined to the non-responder subgroup. The development of clinical resistance in SCLC may be accompanied by loss of specific neuroendocrine features. Immunohistological staining visualizes such a change. This finding should be taken into account in deciding whether an individual patient with recurring SCLC should be given reinduction chemotherapy or not.

In most SCLC tumors properties compatible with a neuroendocrine differentiation can be recognised both in vitro and in vivo using monoclonal antibodies. Similar properties can be recognised in a minority of non SCLC tumors. To investigate whether clinical behaviour of these non-SCLC tumors in which neuroendocrine properties could be recognised mimiced SCLC, clinical data of 141 patients were analysed in Chapter IX. Several parameters which were thought to indicate resemblances between this non-SCLC subgroup and SCLC were assessed. However survival, stage distribution, and the phenotype in metastatic lesions did not support a hypothesis suggesting resemblances in tumor biology between this non-SCLC subgroup and SCLC.

In conclusion this study shows that:

Phenotypic parameters obtained from three SCLC derived cell lines obtained during longitudinal follow-up in one patient only partially reflected the development of drug resistance. We could not recognize the "variant" phenotype in the cell line obtained from a biopsy specimen at second recurrence of the SCLC tumor. Results of the *in vitro* drug sensitivity assay were in agreement with the treatment status of the tumor.

Immunohistochemical staining can be performed in all diagnostic specimens without interfering with normal histological work-up.

Using a monoclonal antibody guided assay, the detection rate of bone marrow metastasis of SCLC increased three-fold. Using this technique it is possible to select those patients amongst "complete responders" according to conventional staging procedures, who have more minimal residual disease. This patient category may be candidate for potentially toxic adjuvant therapies such as radiotherapy or surgery. It may also select patients for adjuvant therapy with lymphokine activated killer cells. In animal experiments it was shown that LAK cell therapy is useful especially if only small amounts of tumor cells have to be killed.

A heterogeneity of neuroendocrine markers expression could be demonstrated in lung cancer using a monoclonal antibody guided staining portocol. In SCLC a subgroup is revealed charactarized by a decreased expression of neuroendocrine differentiation antigens. This subgroup is less responsive to chemotherapy and might emerge during chemotherapeutic treatment of neuroendocrine marker positive SCLC. In non–SCLC, a series of clinical parameters obtained from patients with neuroendocrine markers on their tumors, did not disclose a seperate clinical entity.